Invited Paper

Growth of crystals in optical tweezers

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ABSTRACT

We report here on the use of optical tweezers in the growth and manipulation of protein and inorganic crystals. Sodium chloride and hen egg-white lysozyme crystals were grown in a batch process, and then seeds from the solution were introduced into the optical tweezers. The regular and controllable shape and the known optical birefringence in these structures allowed a detailed study of the orientation effects in the beam due to both polarization and gradient forces. Additionally, we determined that the laser tweezers could be used to suspend a crystal for three-dimensional growth under varying conditions. Studies included increasing the protein concentration, thermal cycling, and a diffusion-induced increase in precipitant concentration. Preliminary studies on the use of the tweezers to create a localized seed for growth from polyethylene oxide solutions are also reported.

1. INTRODUCTION

Optical tweezers represent a powerful non-contact method for positioning and manipulating microstructures. In particular, the optical field can be used to trap small particles, concentrate large macromolecules, and rotate particles under appropriate conditions. For rotation to occur, some sort of anisotropy is required, either in shape or material properties. Protein crystals, such as those of hen egg-white lysozyme, exhibit both shape and material anisotropy, making them of interest for fundamental studies of the interaction of matter with optical tweezers. In this paper, we examine the physics of trapped lysozyme crystals, and also explore the use of tweezers as a possible tool for enhancing crystal growth.

The use of a trapping beam allows suspension of the crystal far from the container walls, repositioning of the crystal, and observation of the crystal during changing conditions. Thermal gradients and thermal cycling can be used to favor growth of the trapped seed and improve the quality of the crystal. In addition, there is a possibility of inducing nucleation of crystals preferentially in the beam, if the crystal component molecule (e.g. protein) or the precipitating agent is sufficiently large to be affected by the gradient force. We have examined the use of high molecular weight polyethylene oxide (PEO), a precipitant for proteins, for this seeding process.

1.1 Optical trapping and alignment

Optical tweezers have been used for a wide variety of applications since the fundamental experiments of Ashkin on light scattering of small particles in solution [1-4]. Momentum transfer from the light in the presence of gradient fields can overcome Brownian motion, and result in the stable 3-dimensional trapping of objects with a refractive index higher than that of the host medium[5]. Most experiments on optical trapping and manipulation are performed on micron-sized objects, but latex particles as small as 38nm have been trapped[6], and polymer chains with radii of gyration down to 10-20 nm can be aggregated in the optical field[7].

Anisotropy of trapped particles, either due to shape, or due to anisotropic optical properties, leads to orientation effects relative to the trapping beam axis and the plane of polarization of the light. Acicular particles will usually (if they are larger than the beam waist radius) be trapped with their long axis aligned with the beam axis, but as the aspect ratio decreases, the effect of the form birefringence is reduced. In the case of spherical particles, optical birefringence becomes the dominant effect, and the particle aligns with the plane of polarization of the incident light. Rotation of this

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plane (via a half-wave plate), or using circularly polarized light, can impart angular momentum to the particle, allowing optically driven rotary motion. This effect is of interest for the development of microfluidic pumps. Recently, we demonstrated the orientation and rotation of lysozyme crystals in optical tweezers[8].

1.2 Materials

1.2.1 Sodium chloride (NaCl)

Reagent grade NaCl was used in the deionized water and PEO solutions. Sodium chloride forms cubic crystals, both crystalographically and in terms of morphology. The expected equilibrium orientation of crystals in optical tweezers is for the body diagonal to align with the axis of the optical beam.

1.2.2 Lysozyme crystals

Lysozyme is a tetragonal crystal in its most common form, and is interesting for optical tweezers studies because the aspect ratio of the crystals is a function of the growth conditions, responding to supersaturation ratio and pH. The optic axis lies along the crystallographic c-direction. We define the aspect ratio as the length of the crystal along the c-axis from point to point, divided by the length of the (110) face (distance AB in figure 1), since these are the easiest distances to measure in photomicrographs. When the crystals are grown with a large aspect ratio, they all align with the c-axis along the beam axis, and no rotation is possible. However, when the crystals are grown with a small aspect ratio (figure 6), the optic axis will tend to be close to perpendicular to the beam axis, and effects of the birefringence can be observed. In figure 1, the morphology, crystallographic axes, and points used in section 3.2.2 are shown.

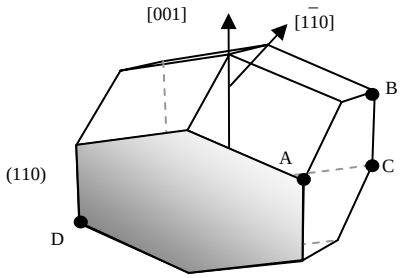

Figure 1 *Crystallographic axes and planes of lysozyme, with points used to define distances used in section 3.2.2*

This flattened morphology is typical for a crystal grown at high supersaturation or high pH conditions. Elongation along the c-axis normally occurs with growth at low pH and low supersaturation ratios. Figure 2 shows micrographs of crystals used in this study. In general, crystallization of proteins involves the use of a precipitation agent such as a salt, or a polymer, such as polyethylene glycol. It has been shown that increased numbers of crystals are observed to grow in solutions that have been aged, due to the increase in the number of heterogeneous nucleation sites[9].

The volume and orientation of the crystals was determined using a MATLAB® routine that generates a rotatable, resizable cage that overlies a micrograph. A graphical user interface permits matching of the cage to the underlying image, and returns the volume of the crystal and orientation angle of the c-axis with respect to the axis of the trapping beam[10].

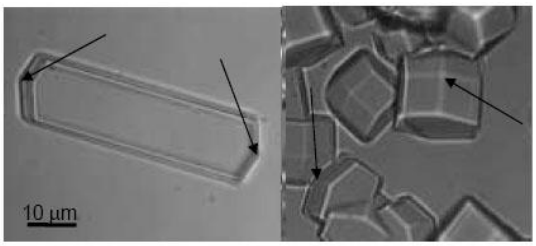

Figure 2 *Photomicrographs of lysozyme crystals. Arrows indicate the ends of the c-axis*

1.2.3 Polyethylene oxide (PEO)

Polyethylene oxide (known as polyethylene glycol in lower molecular weight ranges) from Alfa Aesar, with a molecular weight of 10^6 Dalton (Da) was used in these studies. Polyethylene glycol is used as a precipitating agent for the crystallization of proteins[11], with the best results obtained for molecular weights in the range of 6kDa-20kDa, at low ionic strengths. Polyethylene oxide becomes insoluble in water at high temperatures, displaying a lower critical solution temperature (LCST, cloud point) near 100ºC. Addition of salts reduces the LCST, as does an increase in molecular weight [12]. The radius of gyration of PEO(MW 10^6) in dilute solutions is 15.7 nm, from $R_g = (M^{0.57})/ (45)$ [13], large enough for optical manipulation.

1.3 Polymer aggregates in optical tweezers

Photon pressure has been used to form aggregates of carbazolyl-containing copolymers, using 250 mW of laser tweezers power at a wavelength of 1064nm[6]. There are a variety of man-made and natural molecules which can be manipulated in an optical field. Simple polymers, such as polyethylene oxide, with a molecular weight of 1 x 10^6, has a radius of gyration large enough to be manipulated, and large globular proteins, such as earthworm hemoglobin (30 nm) are also candidates for direct trapping. Lysozyme (1.5nm), when complexed with fluorophores, has been aggregated in the presence of an optical beam [14].

2. EXPERIMENTAL

Experiments were performed on the trapping, orientation, growth and regrowth of NaCl and lysozyme crystals within the tweezers, and on the formation of polyethylene oxide aggregates, as potential seeds for crystal nucleation. The optical system used for measurement, as well as sample preparation conditions, are described below.

2.1 Optical tweezers system

All experiments were performed using a linearly polarized beam from a Yb-doped fiber laser operating at 1070 nm. The light was coupled into a 100x oil-immersion objective with a numerical aperture of 1.3, after reflection from a dichroic mirror which allowed recording of the trapping events on a CCD camera. Illumination for the camera was provided by a white light source placed above the sample. A half-wave plate (HWP) for changing the polarization plane, or a polarizer for controlling the laser power on the benchtop, rather than at the power supply, could be placed in the beam. A low-power HeNe laser was aligned collinearly with the infrared beam for observation of scattering events on the same camera. This allowed indirect observation of trapping and aggregation of particles smaller than the resolution limit of the optical system. The particles could either be identified via changes in the diffraction patterns due to Brownian motion, or by blocking the central portion of the HeNe reflection allowing only rays which are scattered at angles larger than those of the incident beam to reach the detector. For the second method the incident HeNe laser beam did not fill the back aperture of the objective, thus a beam block could be used to detect the scattered light only. The setup is shown in Figure 3, with only the axis of the HeNe beam shown for clarity.

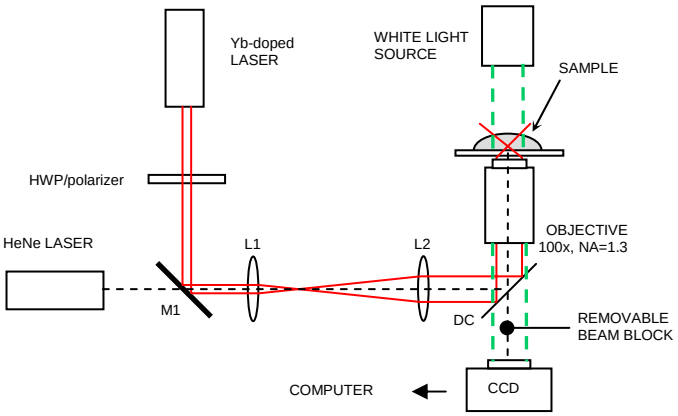

Figure 3 *Experimental setup used for crystal growth measurements*

The temperature of the sample could be varied using thermoelectric elements (not shown) between the stage and sample, and between the microscope body and objective. The temperature range accessible with the setup was +10 /-5 °C from ambient temperature.

2.2 Sample geometries

Three different sample types were used in the experiments described here - open drops, drops in closed volumes, and drops with cover slips placed so that the rim of the drop was exposed through the gap maintained by the gasket. The closed volumes were used for experiments such as temperature variation, to eliminate evaporation during the experiment, while the open drops, and partially exposed drops allowed different rates of evaporation for those experiments based on vapor diffusion. For the closed drops and the partially exposed drops, a 0.8 mm thick silicone gasket assured a fixed sample height and a reproducible evaporation surface. Use of the cover slips greatly reduced concentration driven currents within the samples, and prolonged the evaporation time. Figure 4 shows the configuration of the partially exposed samples. Drops were placed so that the meniscus was not in contact with the edge of the cover slip. Sample volumes were between 20 and 100 microliters.

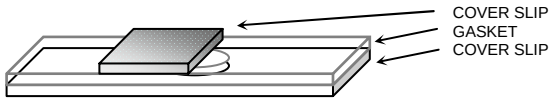

Figure 4 *- Sample configuration for slow evaporation*

2.3 Sample preparation

All solutions used in the experiments, except the lysozyme seed stock, were filtered before use. For NaCl, 0.22 μm polyethersulfone filters were used. For polyethylene oxide (PEO), we used 0.45μm Teflon® (PTFE) filters, as the concentration of PEO, as determined by fluorescence measurements, was substantially reduced after filtration through the smaller pores. Seed crystals were prepared prior to introduction of the sample into the tweezers for NaCl and lysozyme growth/regrowth experiments. NaCl crystals were crushed between two glass surfaces and introduced into the saturated solution to serve as seeds for growth demonstrations. Lysozyme was prepared by the batch method, dissolving as-purchased lyophilized protein powder to a concentration of ~480 mg/ml in a 0.6M acetic acid solution. This protein solution was combined with basic (pH 7 or 9) 5% NaCl solutions to produce a shower of crystals under agitation.

PEO solutions were made in deionized water, stirred vigorously, allowed to sit overnight and stirred again. When all visible inhomogeneities were gone, the solutions were characterized by fluorescent emission in the range 300-450 nm, filtered, and remeasured to assure that the net content of PEO was not substantially reduced. The experiments on PEO were performed by starting with a saturated solution of NaCl in a 2.5mg/ml solution of PEO in deionized water. Deionized water was used to dilute the sample with water in the ratio PEO/NaCl 2.0/1.4 H_2O. For some experiments, the solution was then allowed to evaporate slowly to approach the cloud point.

3. RESULTS AND DISCUSSION

We report on orientation effects in both NaCl and lysozyme crystals. We find that the dominant mechanism for orientation in both these systems is the morphology of the crystal. For NaCl, there is no optic axis in the crystal, but for lysozyme, the anisotropy in the optical properties of the material are sufficient to drive the orientation of the crystal through an abrupt orientation change as the crystal aspect ratio is decreased.

Growth of crystals in optical tweezers can be induced through several mechanisms - vapor diffusion resulting in a higher concentration of the solute (and precipitant, if one is used), addition of solute, or temperature changes (most crystal growth occurs with temperature reduction, but some proteins and polymers have a reduced solubility at higher temperatures). In the experiments reported here, the emphasis was on proof of principle. We used NaCl to demonstrate growth by vapor diffusion, lysozyme to demonstrate growth by addition of solute, and lysozyme to show the improvement in crystal quality made possible by cycling the temperature with a trapped seed in the beam.

We were also able to use the technique to assess qualitative differences in the binding between protein crystals and surfaces prepared by different methods. This could be a valuable technique for improving the results of batch grown crystals in the absence of optical tweezers.

The possibility of using optical tweezers to increase the concentration of precipitant locally has been investigated, using PEO. We find that we are able to form aggregates of large molecular weight molecules. For conditions where the PEO is not far from its solubility limit, we find that we are able to form comparatively large aggregates of the polymer.

3.1 NaCl Growth in tweezers

We introduced small seeds of NaCl into a saturated salt solution in the open drop and partially covered drop configurations. For the open drops of 20 microliters, increase of the salt due to evaporation occurs on a 10- 30 minute time scale, and video-microscopy was used to determine the orientation and growth rate of the crystal. The sequence of images is shown in Figure 5 below.

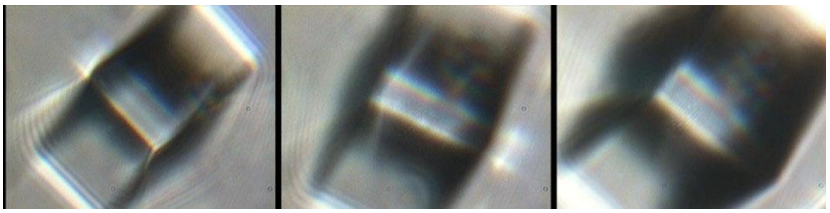

Figure 5 *Images of NaCl growth during vapor diffusion*

3.2 Lysozyme

3.2.1 Concentration changes

We demonstrated growth of a trapped lysozyme seed by addition of a concentrated lysozyme solution to the observation volume in an open drop experiment. The growth rate in this highly supersaturated condition was linear in the initial portion of the growth, with the volume of the crystal doubling in less than one and a half minutes. This corresponds to the addition of approximately 400 μm^3 of lysozyme to the crystal seed. While this rapid growth is unlikely to result in desirable x-ray diffraction properties, it demonstrated the possibility of growth using solute concentration as the driving force for macromolecules. Growth saturated within 5 minutes, as all of the excess lysozyme precipitated out on the suspended crystals and those attached to the substrate surface. Figure 6 shows a series of images demonstrating the growth. This series also shows a change in the orientation of the crystals, discussed below.

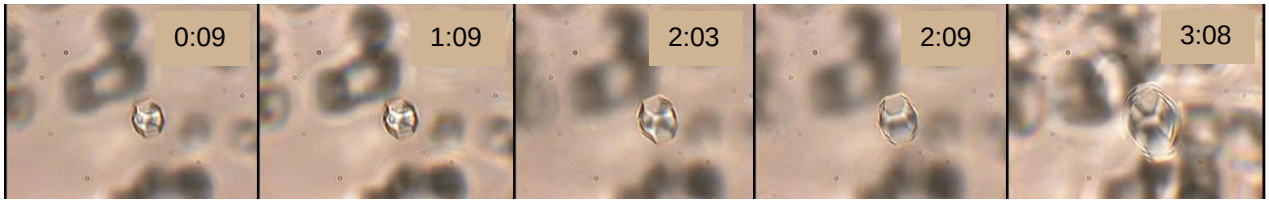

Figure 6 *Growth of lysozyme crystals on addition of protein concentrate*

Attempts to use a diffusive increase in the NaCl concentration (through a microfluidic connection to a high concentration NaCl solution) in a lysozyme drop to promote crystal growth proved to be unsuccessful, due to secondary nucleation.

3.2.2 Orientation effects

The orientation of the crystal in the tweezers is a function of the aspect ratio and birefringence as well as the size of the crystal relative to the beam waist. In the example above, the addition of the lysozyme onto the seed occurs primarily by addition of material to the (110) faces. This results in a reduction in the aspect ratio of the crystal as it grows. If the geometry of the crystal were the only influence on the orientation, one would expect that the longest dimension of the crystal would align with the beam axis.

In figure 7, we hold the [100] distance, and hence the width (AB), constant, and increase the value of the c-axis length linearly. We present the length of the body diagonal (BD) and the angle of the body diagonal relative to the c-axis, as

the aspect ratio changes. The largest distance should determine the direction that aligns with the beam, if one ignores birefringence effects. At high aspect ratios, the cap angle assures that the c-axis length is longest, and the crystals should align with the beam axis. As the aspect ratio is reduced (moving from right to left on the graph below), the body diagonal crosses over, and the crystal aligns as shown in the left frames of Figure 6. There is a gradual change in the orientation of the crystal as the aspect ratio is reduced further. The sudden orientation change evidenced between the frames at 2:03 and 2:09 in Figure 6 (aspect ratio less than one) is not predicted by this model. The change in alignment is induced by the birefringence of the crystal, and is modeled including a full scattering treatment by Singer, *et al.* [15].

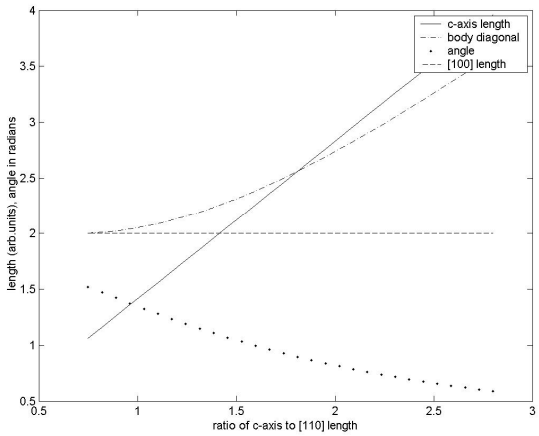

Figure 7 *Lengths through a lysozyme crystal as a function of aspect ratio*

3.2.3 Temperature cycling

In the closed cell configuration, we tested the effects of thermal cycling on the quality of lysozyme crystals. We found that a highly defected crystal could be reduced in volume to a small seed, and then regrown to demonstrate a high degree of crystalline perfection. Figure 8 shows the results of this growth process. The temperature was raised 5 °C, and then allowed to return to room temperature. In the initial images, the irregularly shaped seed is rotated by momentum transfer, but after dissolution to a minimum size, and regrowth, the crystal maintains a constant orientation. Note that the aspect ratio of the crystal does not change during growth.

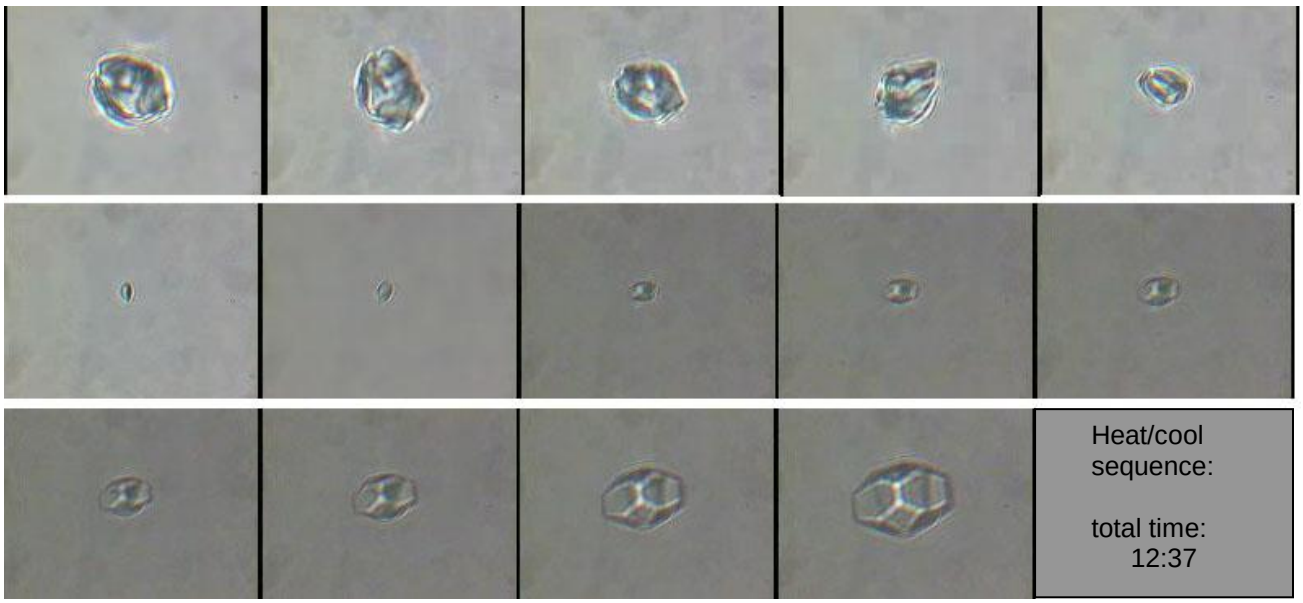

Figure 8 *Dissolution and regrowth of a lysozyme crystal shard, with dramatic improvement in crystal form.*

3.3 PEO

We investigated the possibility of forming aggregates in PEO as a method for seeding inhomogeneous nucleation of crystals. The stability of a PEO solution is determined by the temperature relative to the cloud point (LCST), which can be modified by altering the salt concentration. The LCST can be readily characterized by measuring the transmission as a function of temperature; as the PEO comes out of solution, it forms scattering centers that reduce the overall transmission. For this work, we started with a saturated salt solution, which has a cloud point near room temperature, and diluted it to raise the cloud point to 50 °C, well above the temperatures that could be reached with the tweezers . The dilute solution was formed by combining the PEO/NaCl saturated solution with water in the ratio of 2:1.4.

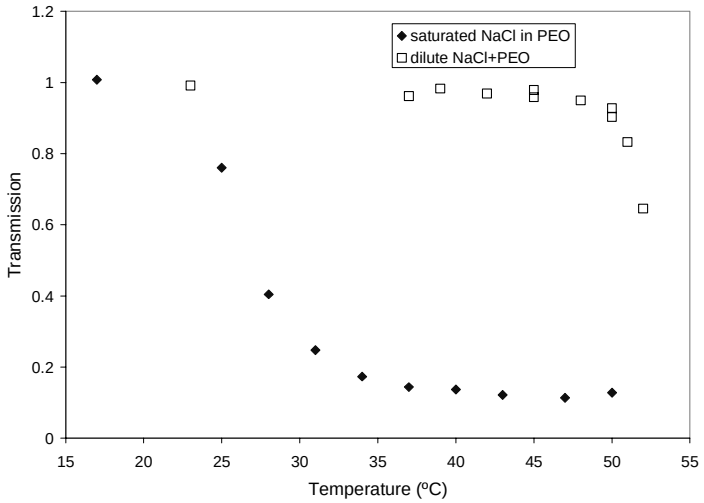

Figure 9 *Transmission of (PEO/NaCl) solutions at 300nm as a function of temperature.*

Initial experiments were performed in closed drops to determine the power dependence of aggregate formation far from the LCST. 34 microliters of dilute solution was placed in a closed volume, and the solution was exposed to high power from the laser. The time to grow from a visible aggregate to 10 microns in diameter was recorded for several power levels, as shown in Figure 10. Although there was substantial scatter in the time to formation of the initial aggregate, there is a clear inverse behavior between power and the growth time of the larger aggregates. Higher powers also resulted in larger values of the maximum size to which the aggregates grew.

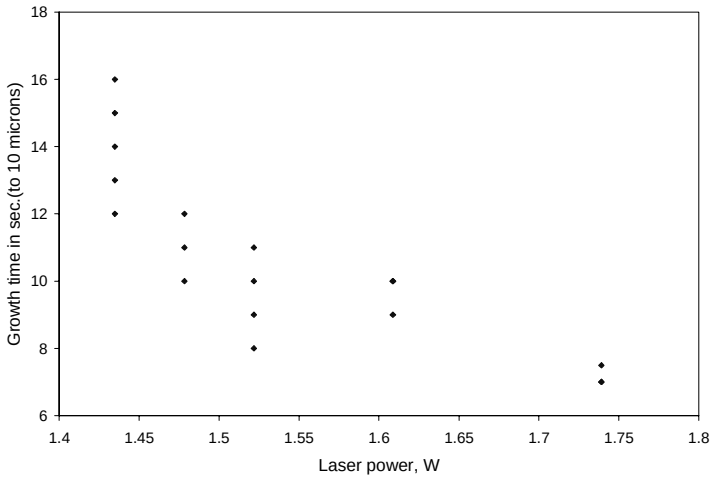

Figure 10 *Growth time of PEO clusters as a function of laser power*

Aggregate formation closer to the cloud point was examined by using the partially covered configuration, which suppresses convection, but allows evaporation of the water in the drop. During the sequence of stills shown in Figure 11, the drop was allowed to evaporate slowly, bringing the cloud point of solution back to the vicinity of room temperature. The laser power (at the sample) was maintained at 1.1 W during the entire growth process. The aggregation of the polymer is most likely a thermal effect in the regime studied here; the power dependence and the increased aggregate size with increasing concentration suggest that the laser creates enough of a thermal gradient to coagulate the polymer chains. This argument is supported by the observation that increasing the overall temperature of the drop using the thermoelectric elements by one or two degrees permits the laser to form a large aggregate with almost no delay. Reduction of power leads to shrinkage of the clusters, and they dissolve rapidly when deposited onto the substrate or released into the solution, as soon as the trapping beam is removed. Experiments are underway to explore the aggregation of the polymer molecules further from the cloud point. The HeNe laser that we have added to the

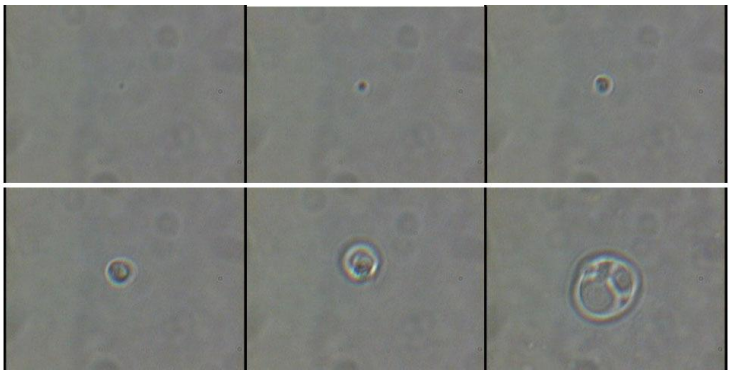

Figure 11 *Image sequence during aggregation of PEO in laser beam*

apparatus gives rise to an off-axis scattering signal that can detect accumulation of material before it becomes visible on the CCD camera.

Conclusions

We have demonstrated the possibility of growing inorganic and protein crystals within an optical trap, and have shown that vapor diffusion and thermal cycling can lead to the growth of crystals in situ. Orientation changes of birefringent crystals are related to the shape and anisotropy of the crystal. Preliminary experiments on the use of PEO aggregation as a potential seed for crystal growth have been performed, and we find that for a molecular weight of 10^6, aggregation is possible. For conditions near the cloud point of the solution, large clusters of PEO can be formed.

Acknowledgements

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References

1. A. Ashkin, "Acceleration And Trapping Of Particles By Radiation Pressure", *Phys. Rev. Lett.* **24**, 156-159 (1970)
2. A. Ashkin, “Applications of laser-radiation pressure”, *Science* **210**, 1081-1088 (1980)
3. D. G. Grier, “A revolution in optical manipulation”, *Nature* **424,** 810-816 (2003)
4. J. E. Molloy and M. J. Padgett, “Lights, action: optical tweezers”, *Contemp. Phys.* **43,** 241-258 (2002)
5. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, S. Chu, "Observation of a Single-Beam Gradient Force Optical Trap for Dielectric Particles", Opt. Lett. 11, 288-290 (1986).
6. K. Svoboda and S.M. Block, ”Optical trapping of metallic Rayleigh particles”, Opt. Lett 19, 930-932 (1994)
7. T.A. Smith, J-I. Hutta, K Sasaki, H. Masuhara, and Y. Itoh, "Photon pressure induced association of nanometer-sized polymer chains in solution", *J. Phys. Chem B* **103**, 1660-1663 (1999)
8. W. Singer, H. Rubinsztein-Dunlop, and U. J. Gibson, "Manipulation and growth of birefringent protein crystals in optical tweezers", *Optics Express* **12**, 6440 (2004)

9. N. E. Chayen, J. W. Radcliffe, and D. M. Blow, "Control of nucleation in the crystallization of lysozyme", *Prot. Sci.* **2**, 113-118 (1993)
10. U. J. Gibson and Y. Kou, "Determination of crystal orientation from micrographs using a MATLAB program", *Appl. Crystall.* **38**, Part 3, 559 (2005)
11. A. McPherson, "Crystallization of proteins from polyethylene glycol", *J. Biol. Chem.* **251,** 6300-6303 (1976)
12. E. A. Boucher and P.M. Hines, "Effects of inorganic salts on the properties of aqueous poly(ethylene oxide) solutions", *J. Polymer Sci. Polymer Phys.* **14,** 2241-2251 (1976)
13. J. Swenson, "Mechanism and Strength of Polymer Bridging Flocculation", *Phys. Rev. Lett.* **81**, 5840 (1998)
14. O.D. Velev, E. W. Kaler, and A. M. Lenhoff, "Photochemical micromachining of lysozyme crystals", *Advanced Materials* **11**, 1345-1349 (1999)
15. W. Singer, T. A. Nieminen, U. J. Gibson, N. R. Heckenberg, and H. Rubinsztein-Dunlop "Orientation of optically trapped nonspherical birefringent particles" *(in preparation)*